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# On nitrogen metabolism in milking cows[1]

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THE CENTRAL PROBLEM in the nitrogen nutrition of ruminants is to what extent the microbial flora of the rumen are able to form protein from ammonium nitrogen. A large number of feeding experiments in which a relatively small part of the proteins has been replaced with urea or some other nonprotein nitrogen compound have been performed in many countries. They have, however, not given any solution to the above-mentioned problem.

The protein requirement of a cow with a high milk production is exceptionally great, because much more protein is secreted in milk during 24 hr than is needed for the maintenance of the cow. Thus it is possible to study the extent of the protein synthesis of the microbial flora of the rumen only with dairy cows. This has been the leading idea since the study of milk production was started in this laboratory 6 years ago with cows fed purified protein-free feed, urea, and small amounts of ammonium salts as practically the sole source of nitrogen. Results of these studies have been described partly in numerous papers (11–18). Oltjen has recently started to study milk production using purified diet (8).

The following scheme (Fig. 1) describes roughly the nitrogen metabolism of ruminants. The proteins of the feed pass partly without decomposing into the abomasum through the proventriculi, after which their utilization is similar to other mammals. Part of the proteins are hydrolyzed in the rumen to amino acids, which will be used partly for microbial protein synthesis, but mainly they will probably be deaminated to ammonia. The digestible nonprotein nitrogen of the feed is utilized as ammonia, which the rumen microbes use for their protein synthesis. Part of the ammonia passes into the blood through the wall of the rumen. In the liver some of it will be used for the synthesis of nonessential amino acids, some is metabolized to urea. Urea can be transported from the blood into the rumen, at the same time decomposing into ammonia. The presence of urea in saliva is well known. The surplus of urea is removed in urine. It seems that the ammonia concentration of the rumen regulates to some extent the transfer of urea from the blood to the rumen. The recent experiments by Várady and his colleagues about the effect of starvation on urea retention and on the $NH_3$ concentration in the rumen of sheep after intravenous administration of urea have confirmed this opinion (10). As said above our problem has been to elucidate to what extent nonprotein nitrogen functions alone without protein nitrogen.

Ten years ago an experiment in which a cow on normal feed was given one dose of ammonium sulfate labeled with $^{15}N$ was performed in our laboratory. The labeling of the amino acids, separated by fractionation after hydrolysis of the milk proteins, was determined (19). All the protein amino acids studied were labeled, but the degree of labeling of the various amino acids differed. The weak labeling of some amino acids suggested that their synthesis may form a bottleneck in protein synthesis. Thus the possibility of developing in the rumen, by adaptation to a test feed, a microbial flora which could synthesize protein from ammonia more effectively than the rumen microorganisms of cows on normal feed and thus make milk production possible seemed to deserve experimental study.

The experiments on protein-free feed with urea and a small amount of ammonium salts as the sole sources of nitrogen were started with one cow in the autumn of 1961 and with another at the beginning of 1962. A very slow adaptation procedure was used. When one of the test cows (Eiru) was given a dose of $^{15}N$-labeled urea after having been on the test feed for 6 months and the labeling of the amino acids of milk protein was quantitatively determined 6.3 hr and 20 hr after the administration of $^{15}N$, the labeling of essential amino acids generally, and especially of some of them, was found to be strongly increased relative to values obtained in experiments with a cow not adapted to the test feed. Another experiment, in which $^{15}N$-ammonium sulfate was given the same test cow after it had been on the test feed for 25 months, gave similar results. Accordingly, a dose of $^{15}N$-urea and a dose of $^{15}N$-ammonium sulfate have the same effect on the labeling of the amino acids of milk protein. The curves in Fig. 2 illustrate the effect of adaptation to the protein-free test feed on the labeling of the essential amino acids of milk protein. The labeling of the different amino acids is expressed as a percent of the labeling of glutamic acid

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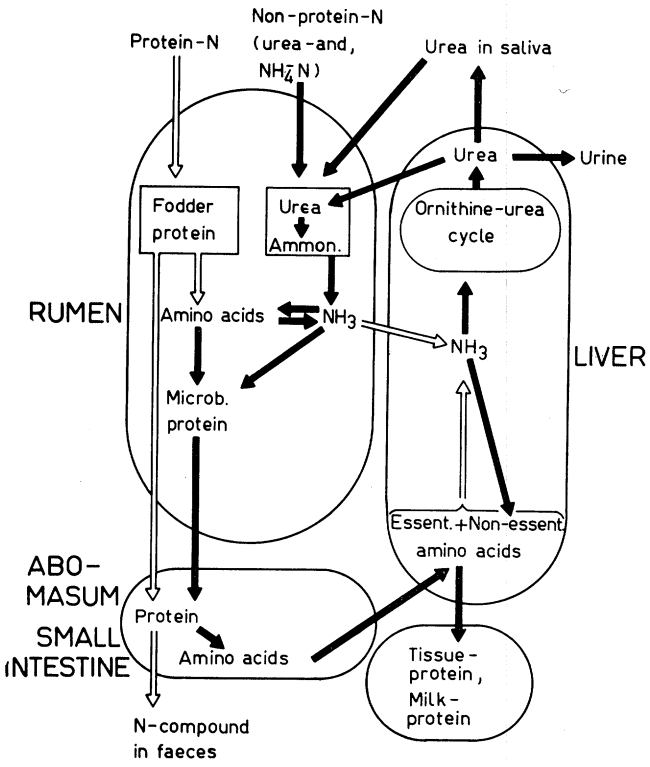



FIG. 1. Scheme of the utilization of protein and nonprotein nitrogen in ruminants.

which always has the highest labeling during the first 10 hr.

Four more cows were included in the experiment in 1963–1964 and later on two more. The adaptation time of 1–2 months seems sufficient, provided that the transference takes place at the end of the lactation period.

The test cows were fed twice a day. The cows producing greater amounts of milk ate their large rations gradually all day long and regulated in this way the intake of urea. There was thus no need to divide the feed repeatedly into small portions.

The test feeding was made up of the components as shown in Table 1.

Nitrogen-containing impurities are present in our test feed in such a small amount that the urea and ammonium nitrogen account for 99.5 % of the total nitrogen.

Different test cows were given briquettes, cellulose-rich paste, and cellulose strips in different proportions according to appetite. Thus the feed of one cow differed from the feed of another to some extent. Roughly speaking it can be said that the carbohydrate of the test feed was starch, cellulose, and sucrose in the following proportions: 50–60 % potato starch, 20–30 % $\alpha$-cellulose, and 17–23 % sucrose. The success of the urea feeding is decisively dependent on the composition of the carbohydrates fed. The proportion of starch or other rapidly digestible polymer carbohydrates in the feed probably cannot be reduced very much without an accompanying drop in milk production.

As seen in Table 1 the nitrogen content of the feed has been raised considerably during the experiment. The largest amount of urea (ammonium nitrogen included) during the first 2 years was only a little more than 400 g/day. As investigations on the rumen contents and blood showed, however, that ammonia had not accumulated in the rumen to an injurious degree and that the ammonia content of the blood had not risen alarmingly, the amount of nitrogen in the feed was raised to such an extent that the cows, weighing about 450 kg, could receive even 600–680 g urea/day corresponding to 1,200–1,360 g digestible crude protein. This addition has raised the milk production very much and it has totally removed those symptoms of probable nitrogen deficiency which were observed when the low-nitrogen feed was used. The hairy coat of the fore part of the legs of the cows, especially that of the hindlegs, thinned, or in some cases disappeared, after about 2 months from calving, and regrew after the milk production had fallen to 5–7 kg/day. On the basis of our many years experience there is no danger of too high urea rations provided that the feed contains enough carbohydrates of good digestibility.

To improve rumination we used rye or wheat straw at first. However, as straw was not included in our program, we tried to omit it by feeding the cows cellulose strips impregnated with silicic acid, which in turn was gradually replaced by polyethylene pellets. Without these pellets the cows ruminated about 1 hr in 24 hr; 400 g pellets/day approximately doubled the rumination time.

The curves of Fig. 3 show how greatly the milk yield has risen since the amount of urea in the feed was in-

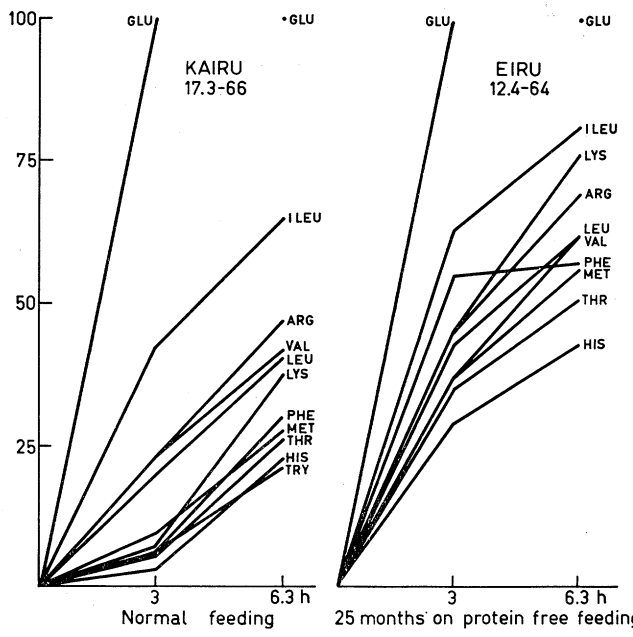



FIG. 2. The $^{15}N$ labeling of the essential amino acids of the proteins of milk 3 and 6.3 hr after the cows had been given a single dose of $^{15}N$-ammonium sulfate. Results are given as percentage of the labeling of glutamic acid, since this amino acid is always labeled most rapidly. On the left are the results of a cow on normal feed, on the right those of a cow which had been on test feed for 25 months. Determinations by M. Kreula and T. Moisio.

TABLE 1. *Composition of the components of the test feed*

*1*) Composition of the briquettes, about 9 g each

| | | 1962–1964 (%) | 1965 (%) |
|---|---|---|---|
| α-Cellulose powder | | 9.5 | 9.9 |
| Starch | | 57.0 | 52.9 |
| Sucrose | | 20.9 | 23.1 |
| Mineral salt mixture | | 8.2 | 8.9 |
| Urea (94%) + ammonium salts | (6%) (calc. as urea) | 4.4 | 5.2 |
| | Total | 100.0 | |
| | Water content | 15.0 | |

*2*) Composition of the wet cellulose-rich paste

| | | 1962–1963 (%) | 1964– (%) |
|---|---|---|---|
| α-Cellulose powder | | 60.3 | 57.3 |
| Starch | | 19.5 | 16.4 |
| Sucrose | | 12.1 | 12.2 |
| Mineral salt mixture | | 7.1 | 8.8 |
| Urea | | 1.0 | 5.3 |
| | Total | 100 | 100 |
| Mixed with water. | Water content | 75 | 75 |

At need, more urea could be mixed with the paste or the sugar-free briquette powder which was occasionally used.

*3*) Cellulose strips, 75% water, urea 4.0% + mineral salt mixture 3.0% in dry matter.
*4*) Vegetable oils, 50–130 g/cow per day.
*5*) Vitamins A (100,000 IU) and $D_2$ + $D_3$ (20,000 IU), from 1.1.65 also vitamin E.
*6*) Mineral salt mixture.

creased in 1964 and 1965. So far, the highest production of standard milk per year has been on an energy basis 4,217 kg, and on a protein basis 4,284 kg; the highest production for a prolonged lactation period has been on an energy basis 5,484 kg, and on a protein basis 6,013 kg (*cow 5*). We have used as standard milk, milk containing 4.0% fat, 3.2% protein, and 4.9% sugar (684 kcal/kg milk).

The daily intake of test feed was at most 11 kg dry substance corresponding to nearly 10 feed units. A daily production of about 17–18 kg standard milk is thus theoretically possible. This is mostly reached, and often exceeded, when body reserves are partly used for milk production.

It is characteristic of high yielding test cows that they have become pregnant only after several services. The estrus of all the test cows has been regular and the reason for the delay in fertilization of the high-producing test cows is so far unknown. The addition of vitamin E to the test feed has not improved the fertilization, according to the results so far.

The calves have been strong and vigorous, weighing on the average 36 kg. They grew steadily on purified feed with urea as the sole source of nitrogen since the 5th month, reaching the weight of 250–290 kg when 1 year old. However, on the basis of our results the heifers did not develop into cows on such a feed, because they died suddenly when 1–2 years old. It is only since the use of α-tocopherol has been started that we have managed to rear milk cows from heifers.

The observations on the rapid utilization of ammonia in the rumen of the cows adapted to the test feed could be explained only by supposing that the microbial flora of the rumen content had been effectively adapted to the utilization of ammonium nitrogen. Investigations in our laboratory concerning the microbial flora of the rumen have shown that the protozoa in the rumen of the adapted cows have generally disappeared, while the number of bacteria has risen enormously (Fig. 4 and Table 2).

It is probable that the bacterial strains which grow well with $NH_4$-N without amino acids, vitamins, or other specific growth factors are the most important in the bacterial flora of the rumen of the test cows. There are, however, in the ruminal flora even bacteria which need specific factors for their growth. These can be important for the effective function of the rumen. Regarding symbiotic growth, I refer to the fundamental work of

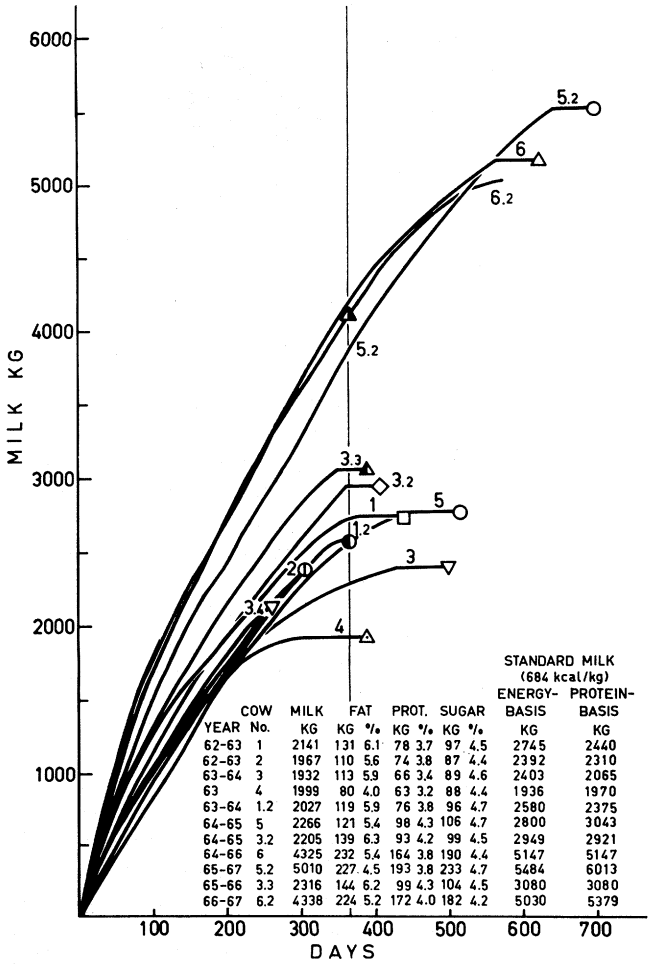



FIG. 3. Milk production of test cows on experimental feed. The milk yield was calculated both on the basis of energy (standard milk, 684 kcal/kg milk) and of protein (standard milk, 3.2% protein). The large numbers on the curves refer to the test cows, the small numbers to the lactation periods during the experimental feeding (for example, 3 = *cow 3*, first period; 3.2 = *cow 3*, second period); the end points of the curves represent the calving times. Annual milk yields may be seen from the curves. Notice the ver similary milk production of *cow 6* (Metta, 12 years old, 1967) during two successive lactation periods.

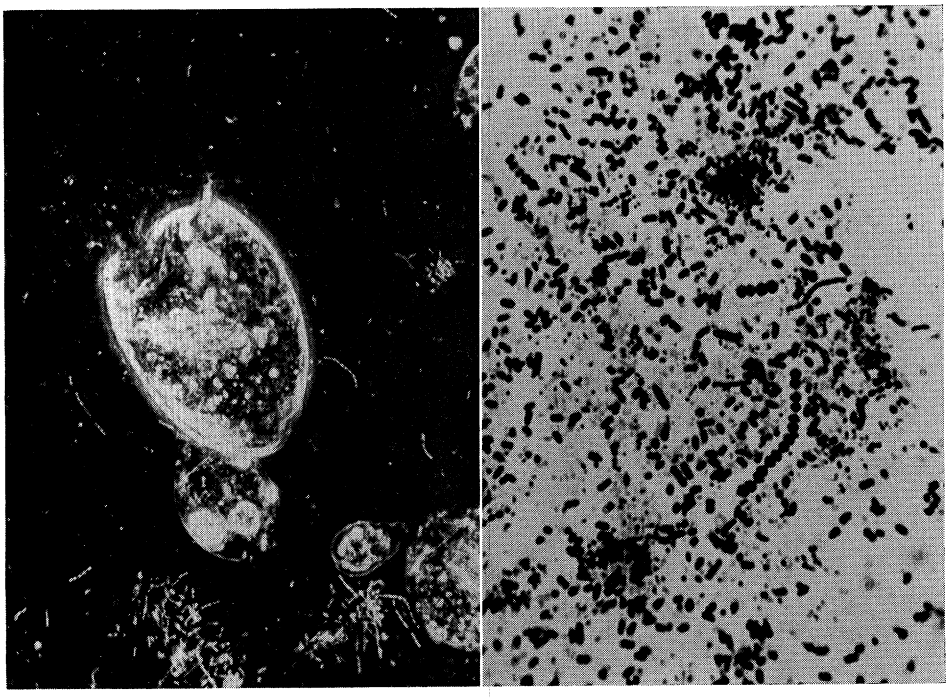


FIG. 4. Microscopic pictures of the rumen contents of cows on normal feed and test feed. *Left-hand figure:* normal feeding. Plenty of big and small protozoas besides bacteria. × 430. Photo M. Lampila. *Right-hand figure:* test feeding. Protozoa were not observed microscopically with low magnification. In the picture masses of bacteria of different types. × 1,000. Photo S. Mäkinen.

TABLE 2. *Microbial counts, urea and ammonia concentrations found in the rumen contents of test and normally fed cows*

| | Time of Sampling, After Feeding | Protozoa per ml | Bacteria per ml | Urea, mg/100 ml | Ammonia, mg/100 ml |
|---|---|---|---|---|---|
| Test cows | | | | | |
| Metta (*no. 6*) | 10 min | 0 | $3.1 \times 10^{10}$ | 17.5 | 32.9 |
| Metta | 100 min | 0 | $4.3 \times 10^{10}$ | 0.4 | 4.3 |
| Jairu (*no. 5*) | 3 hr | 0 | $3.0 \times 10^{10}$ | 13.4 | 9.0 |
| Jairu | 7 hr | 0 | $1.2 \times 10^{10}$ | 2.6 | 1.6 |
| Normally fed cows | | | | | |
| Asteri | 3 hr | $7.9 \times 10^{4}$ | $3.6 \times 10^{9}$ | 0 | 22.4 |
| Mili | 3 hr | $4.4 \times 10^{4}$ | $2.3 \times 10^{9}$ | 0 | 16.6 |
| Muoti | 3 hr | $1.0 \times 10^{5}$ | $3.7 \times 10^{9}$ | 0 | 22.7 |

Nurmikko in our laboratory on the lactic acid bacteria 15 years ago (7). He showed conclusively in his dissertation that different strains of lactic acid bacteria can feed one another with different growth factors when they are grown in the same medium, because the factors synthesized in the bacterial cells are partly excreted into the medium. This means that in mixed cultures different strains can grow in a medium that is much simpler than that needed by the same strains in pure cultures (Fig. 5). The symbiotic growth is probably general in microbial associations, and the microbial flora of the rumen contents may be one of the most complicated examples of this type of system.

In the amino acid composition of the hydrolyzate of the total protein of the rumen contents of test cows and of control cows on normal feed, systematic differences could clearly be found only regarding $\alpha, \epsilon$-diaminopimelic acid, a characteristic constituent of the cell wall of many bacteria. It was regularly present in much higher amounts in the hydrolyzate of the rumen protein of test cows than in that of normally fed cows. There is some evidence also for the higher concentration of $\gamma$-amino butyric acid and ornithine, both components of the bacterial cell wall, in the hydrolyzate of the rumen protein of test cows.

Studies on the composition of the nitrogenous compounds of the rumen contents have shown that the ammonia in the rumen does not increase to values which should be harmful; besides, it falls rapidly after feeding (Fig. 6) and is then lower than on normal feeding. The highest amount of ammonia we have found in the rumen contents of test cows just after the feeding, has been not quite 40 mg/100 ml. During the highest and most rapid intake of feed the amount of ammonia will probably rise above this.

The composition of the nitrogenous compounds of the feces of the test cows has also been studied in our laboratory. In experiments with rats, McNaught et al. (5) found the digestion coefficient of the protein in a bacterial fraction grown in strained rumen liquor with urea and carbohydrate supplement to be 74. If this coefficient is the same in ruminants, the main part of the nitrogen of the feces of the test cows should be indigestible bacterial protein. In fact, among the normal amino acids which are found in the hydrolyzate of the protein fraction of the feces of the test cows, $\alpha, \epsilon$-diaminopimelic acid is present in much higher amounts than in the same fraction from cows on normal feed (15). This is in agreement with findings for the amino acid composition of the proteins of the rumen contents. Although not all rumen bacteria contain diaminopimelic acid in their cell wall "proteins," the high content of this amino acid in the protein fraction of feces strongly supports the implication of other results, that bacterial protein synthesis is greatly enhanced in the

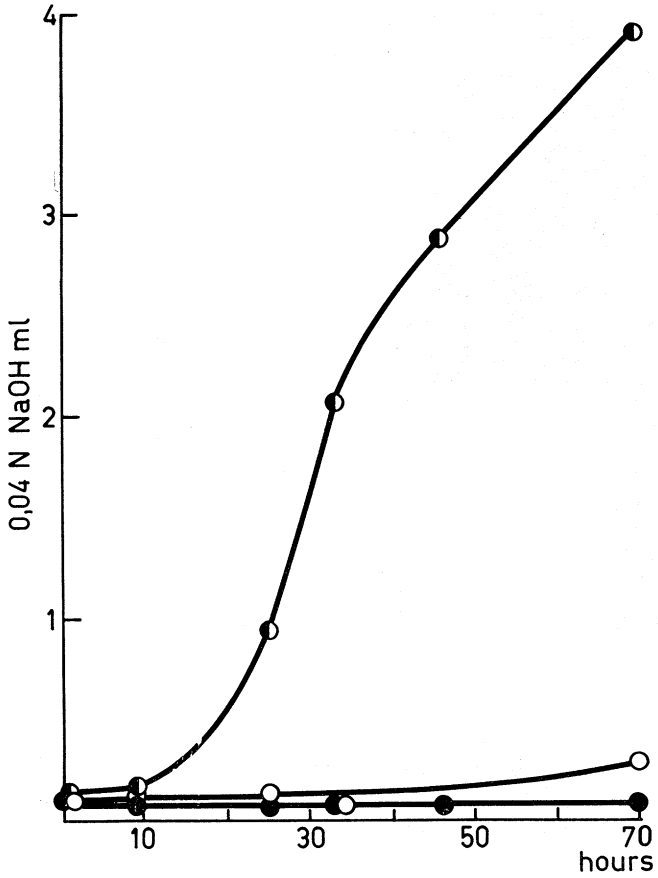



FIG. 5. Growth of *Lactobacillus arabinosus* 17-5 and *Streptococcus faecalis* R in symbiosis. Basal medium without phenylalanine and folic acid. ● *L. arabinosus* 17-5 (phenylalanine-requiring strain). ○ *S. faecalis* R (folic acid-requiring strain). ◐ *L. arabinosus* 17-5 and *S. faecalis* R together.

rumen of the test cows. The ammonia, urea, and creatinine nitrogen analyses in urine are shown in Table 3.

Because the mammary gland receives the raw material for the synthesis of different components of milk from the blood, knowledge of the composition of the blood of the test cows is important.

The free amino acids of the blood form only a very small part of the nitrogenous compounds of the blood, but they are decisively important for the formation of the proteins of milk in the mammary gland. The concentrations of most of the free amino acids, particularly the essential amino acids, in the plasma and the whole blood of the lactating test cows are lower than those in plasma and blood of the control cows on normal feed (Fig. 7). The relative decrease in the concentration of free histidine in plasma is greater, in cows on the test feed, than the decrease for any other amino acid. The histidine content decreases some weeks after calving, remains low for several months, and then rises again to some extent while the milk production is decreasing. At its lowest level, the free histidine content of the plasma can be about 20 % of the corresponding values for normally fed cows.

Low concentrations of free amino acids are found in the plasma of the lactating test cows; the concentrations of histidine and many other amino acids in the plasma of dry test cows and of heifers is much higher.

The very low ammonia concentration in the blood of the test cows is in accordance with the great ability of the adapted ruminal flora to utilize ammonium nitrogen. Whether the ability of the liver to utilize ammonium nitrogen has increased is not known but this is possible. Holzschuh and Wetterau have recently considered this to be the case (1).

The hemoglobin content of the blood of the test cows decreases during the highest milk production more than that of normally fed cows (Fig. 8). It seems, however, that the difference between the test cows and normally fed cows is smaller when more urea is used in the feed. Since the content of free histidine in the plasma of the test cows is very low, and histidine may form a bottleneck in protein synthesis in cows on the test feed, it may be that the primary reason for this reduction in hemoglobin is the deficiency of histidine during the postcalving period, when the protein requirement is greatly increased because of milk production.

The amino acid composition of the whole-blood protein of the test cows was similar to that of normally fed control cows. In electrophoretic studies carried out in this laboratory on the serum proteins of several blood samples of the test and control cows, only slight differences in the various protein fractions in samples from individual cows could be observed, and the differences in the results for test and control cows were slight.

Milk produced on a protein-free feed is called zero milk (0-milk) in our laboratory. Its composition has been the object of many-sided research. We have been especially interested in comparing the proteins of the 0-milk with those of milk produced on normal feed (9). All the methods we have used—DEAE-filtration and various gel

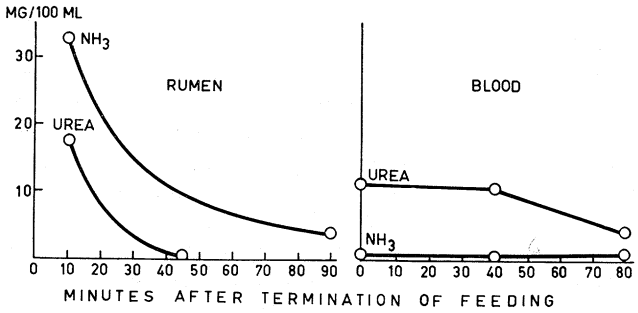



FIG. 6. Ammonia and urea content in the rumen and blood of a test cow.

TABLE 3. *Analyses of some nitrogen compounds in the urine of cows*

| | Total N % | $NH_4$-N, %/total N | Urea-N, %/total N | Creatinine-N %/total N |
|---|---|---|---|---|
| Normal feeding (*farm 1*) | 1.13 | 3.0 | 30.3 | 6.0 |
| Normal feeding (*farm 2*) | 1.27 | 0.6 | 33.2 | 4.4 |
| Protein-free test feeding | 0.48 | 2.1 | 49.6 | 6.8 |
| Low protein, high urea feeding | 0.72 | 0.7 | 40.3 | 5.8 |

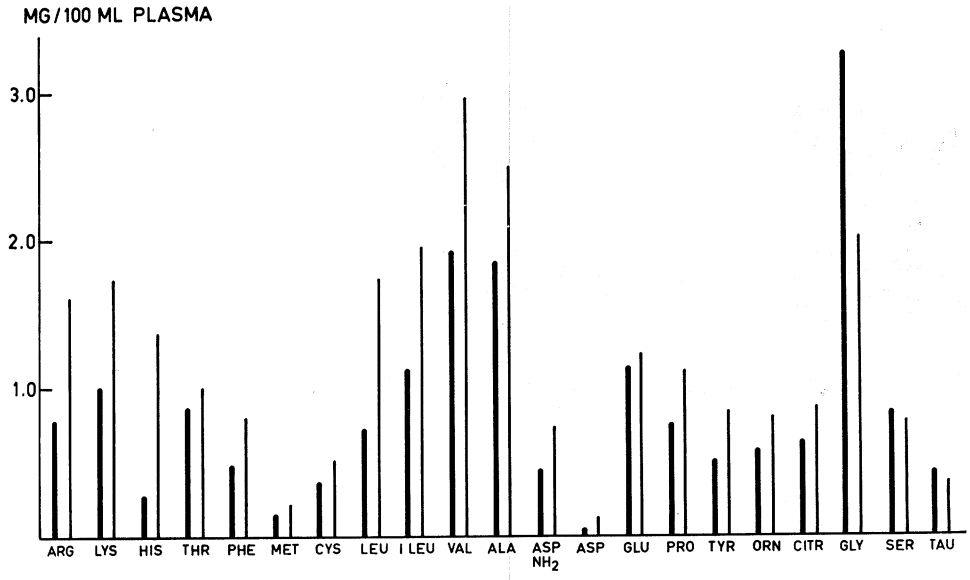



FIG. 7. Amount of free amino acids (mg/100 ml) in the blood plasma of milk-producing cows. *Thick columns:* test cows fed on purified protein-free feed. *Thin columns:* cows on normal feed.

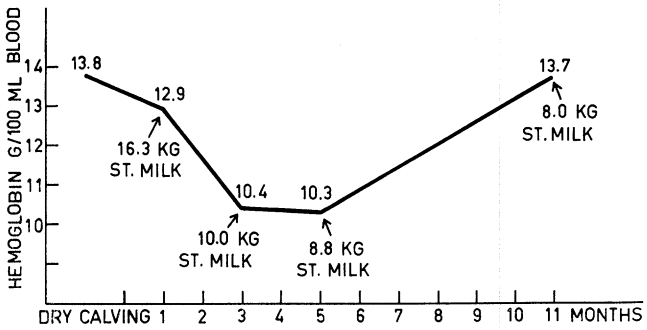



FIG. 8. Hemoglobin content of the blood of test *cow 5* (Jairu) 1, 3, 5, and 11 months after calving.

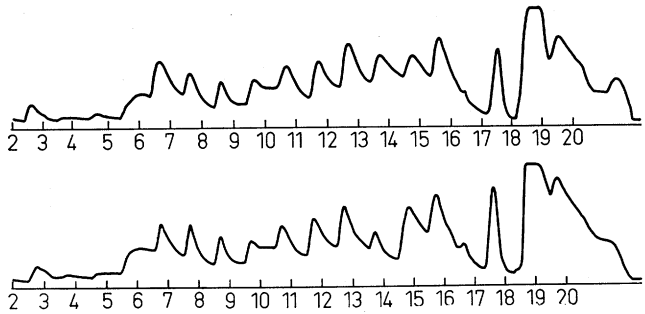



FIG. 9. Fractionation of the milk proteins on a DEAE-column. *Above:* milk of the test cow Eiru (no. 1), *below:* milk of cows on normal feeding.

electrophoreses—have led to the result that no certain differences due to the feed have been observed (Figs. 9, 10, 11).

According to Larson and Gillespie over 90% of the total protein in milk is synthesized in the mammary secretory cells from free amino acids (4). The synthesis of these proteins is carefully controlled by the genes. If some amino acids are not present in sufficient amount, protein synthesis decreases. The second small group including the immune globulins is, according to the authors, blood proteins entering milk from the bloodstream.

The flavor of 0-milk is to such an extent similar to that of normal milk that it is not easy to distinguish between these milks in organoleptic tests, provided no off-flavor due to the feed used occurs in normal milk. I have been especially interested in this problem, because earlier it has not been known to what extent milk flavor is due to the feed and to what extent the flavor substances of milk are formed in the cow's organism. The flavor of 0-milk and normal milk has been studied gas chromatographically and mass spectrometrically in our laboratory for many years (14). The compounds having a major role in milk flavor are found both in 0-milk and normal milk.

When basic knowledge about the milk production of cows on purified, protein-free feed had been obtained, it was probable that much larger amounts of protein could be replaced with urea, when nonpurified feed is used, than was supposed earlier. The comparison of feed low in digestible true protein supplemented with large doses of urea was of greatest interest. A feeding experiment using rations containing about 20, 40, and 50% digestible true protein of the digestible crude protein was arranged. To reach such a low content of digestible true protein in the feed as 20% is difficult. We used in the daily ration fresh potatoes, dried sugar beet pulp, and dried hemicellulose syrup prepared from wood. Digestible urea nitrogen formed in this diet 65% of the digestible crude protein. It was already easier to arrange the feed rations containing 40% or more digestible true protein of the digestible crude protein, because oats and barley could then be included in the feed rations. Table 4 shows the composition of the daily feed ration of *cows 20–25* during the period of high milk production after calving (*20.1* and *20.2* mean *cow 20* after the first and second calving, respectively). In Fig. 12 the annual milk yields which have been obtained using the feed combinations described in Table 4 have been shown with columns. For

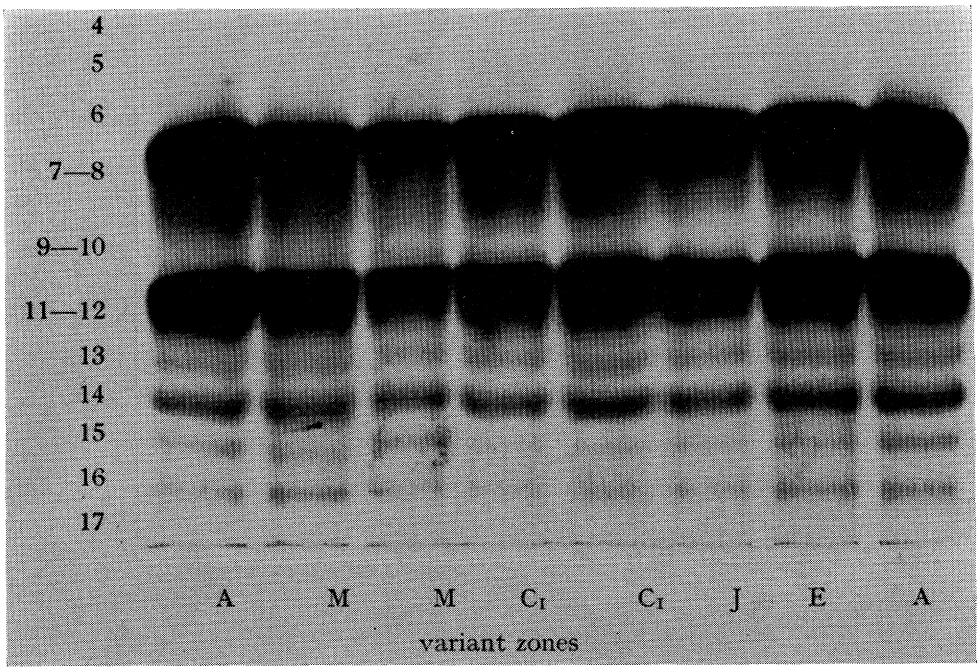



FIG. 10. Starch gel electrophoresis of acid caseins from milks of the test cows Aino (A), Metta (M), Jairu (J), Eiru (E) and control milk I ($C_I$). Samples were taken in December 1964. Zone designations are according to Neelin (6).

TABLE 4. *Feeding during the highest milk production per day*

| Feed | Cow Number | | | | | | |
|---|---|---|---|---|---|---|---|
| | 20.1 | 20.2 | 21.1 | 22.1 | 23.1 | 24.1 | 25.1 |
| Potatoes, kg (d.m.20%) | 22.5 | 20.0 | | | | | |
| Sugar beet pulp, kg (d.m.90%) | 3.5 | 7.0 | 4.7 | 4.7 | | | |
| Oats, kg (d.m.90%) | | | 5.5 | 5.0 | 6.0 | 8.5 | 6.0 |
| Barley, kg (d.m.90%) | | | 1.5 | 2.0 | 4.0 | 2.0 | 1.5 |
| Hemicellulose, kg (d.m.96%) | 2.3 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Silage, kg (d.m.20%) | | | | | | | 16.0 |
| Hay, kg (d.m.80%) | | | | | | | 2.0 |
| Straw, kg (d.m.80%) | 1.5 | 0.3 | 1.0 | 1.0 | 2.0 | 2.0 | |
| 0-fiber, kg (d.m.100%) | 2.3* | 1.0† | 0.4* | | | | |
| Urea, g | 440 | 560 | 440 | 440 | 350 | 370 | 340 |
| Salt mixture, g | 750 | 750 | 400 | 400 | 400 | 400 | 350 |
| Feed units/day | 10.2 | 12.9 | 12.8 | 12.7 | 12.3 | 12.4 | 12.3 |
| Feed units/year | 3,365 | ~3,750 | 3,583 | 3,875 | 3,026 | 3,549 | 3,811 |
| Urea, kg/year | 136.0 | 155.0 | 123.1 | 129.3 | 93.9 | 95.1 | 89.0 |

* 0-fiber of low quality, digestibility about 50%. † 0-fiber of high quality, digestibility 80%. d. m. = dry matter

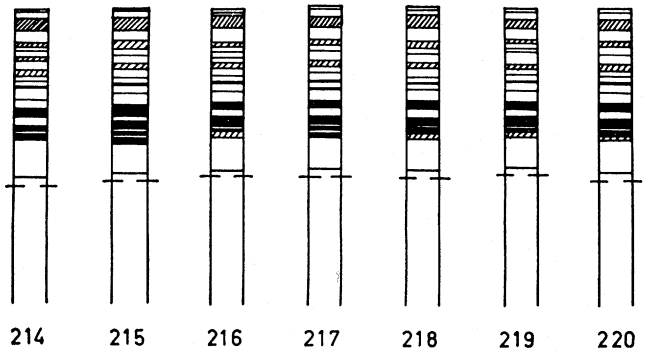



FIG. 11. Drawings of disc electrophoretic patterns of the whey proteins of the test cows, Aino (214, 218), Jairu (215), Metta (216, 219) and control milk I (217, 220). Samples in tubes 214–217 were taken in February and in tubes 218–220 in April 1965.

comparison Fig. 12 also contains the best annual milk yields of the test cows fed on protein-free purified nutrients, with urea and ammonium salts as the sole sources of nitrogen.

Figure 12 shows clearly that when nonpurified feed low in digestible true protein but supplemented with high doses of urea has been used, milk production has risen considerably compared with the milk production of cows on protein-free feed. The increase has been roughly 1,000–1,500 kg milk/year when the feed contained 20% and 40% digestible true protein, respectively. This is due to the higher intake (2–3 feed units) of nonpurified feed containing some protein. The amount of feed eaten depends again obviously on the utilization of the feed in the rumen and other parts of the intestinal canal. As nonpurified feed contains a great number of most differ-

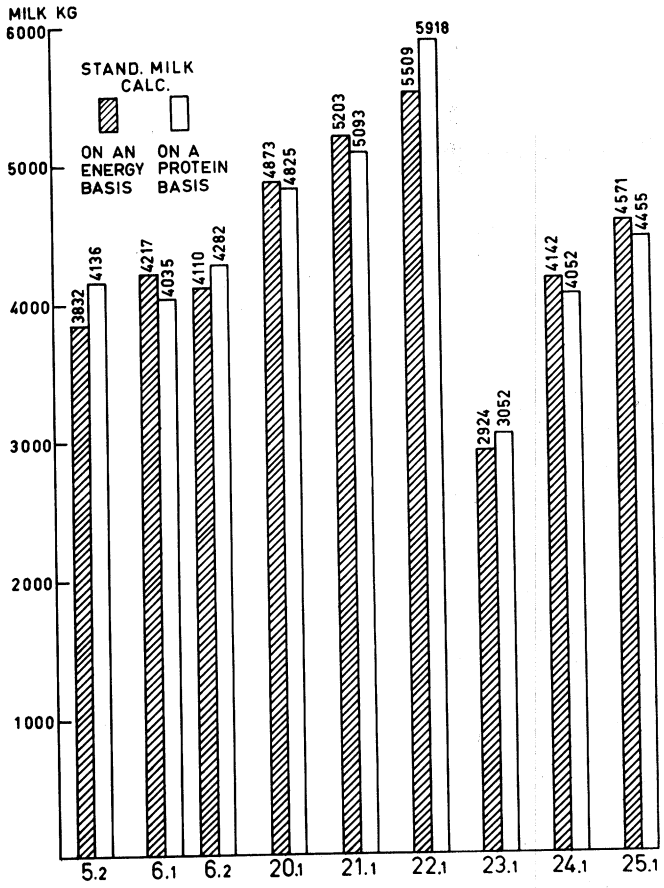



FIG. 12. Milk production of test cows on a protein-free feed (cows no. 5 and 6) and of cows on feed containing about 20, 40, and 50% digestible true protein (*cows 20–25*). 5.2, Second lactation period. 6.1, First lactation period. 6.2, Second lactation period.

| | Cow No. | Urea Fed/ Year, kg | Feed Units, fu/year | True Protein, % of Crude Protein |
|---|---|---|---|---|
| Purified rations without protein | 5.2 | 169.7 | 2,695 | |
| | 6.1 | 160.1 | 2,838 | |
| | 6.2 | 189.3 | 2,798 | |
| Feed with different protein content | 20.1 | 136.0 | 3,365 | 21.1 |
| | 20.2 | ~164.7 | ~3,928 | ~21.2 |
| | 21.1 | 123.1 | 3,583 | 39.7 |
| | 22.1 | 129.3 | 3,875 | 40.5 |
| | 23.1 | 93.9 | 3,026 | 44.6 |
| | 24.1 | 95.1 | 3,549 | 48.7 |
| | 25.1 | 89.0 | 3,811 | 50.5 |

FIG. 13. Amount of free amino acids in blood plasma. Protein-free feed (dark columns), 20% digestible true protein of digestible crude protein (dotted line columns), 40% digestible true protein of digestible crude protein (broken line columns), normal protein-rich feed (white columns).

ent compounds which are not found in purified nutrients, it is difficult to say what substances in the nonpurified feed have increased the intake of fodder. On the basis of blood analyses digestible true protein increases the amount of free amino acids in the plasma when digestible true protein is included in the feed (Fig. 13). The sister *cows 21* and *22* on a feed containing about 40% digestible true protein-N and 53% digestible urea-N of the digestible total-N have given the highest annual milk yields. During the 2nd year the production of *cow 22* is expected to reach 7,000 kg standard milk. *Cows 23* and *24*, the

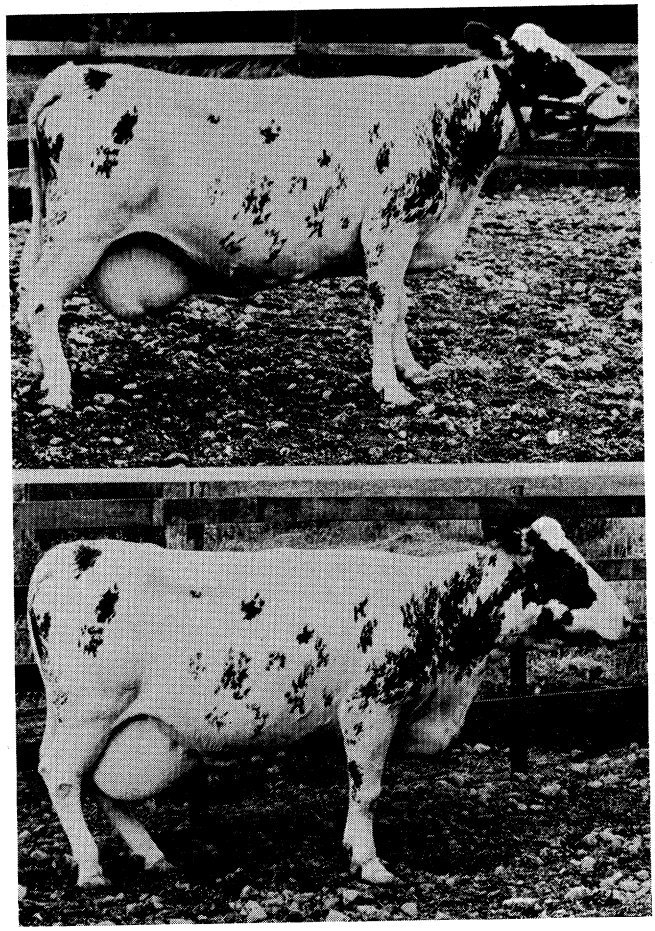
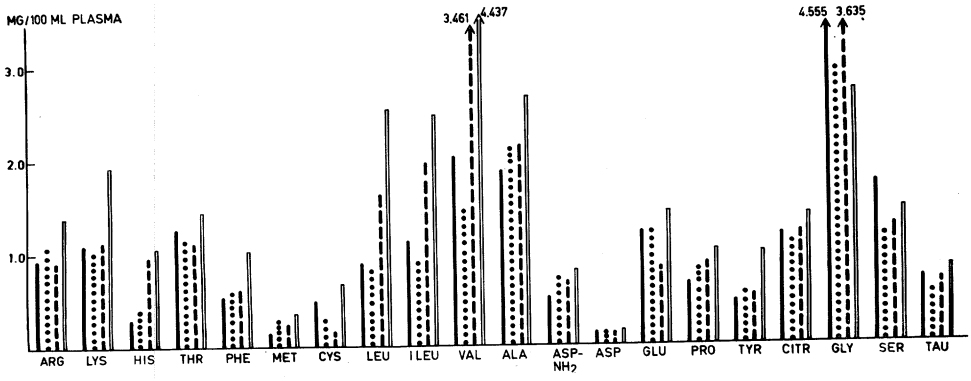


FIG. 14. Test cow Metta (no. 6) after being on the purified, protein-free test feed for 1 year (*top*) and for 3 years (*below*) Age of the cow in the latter case 12 years.



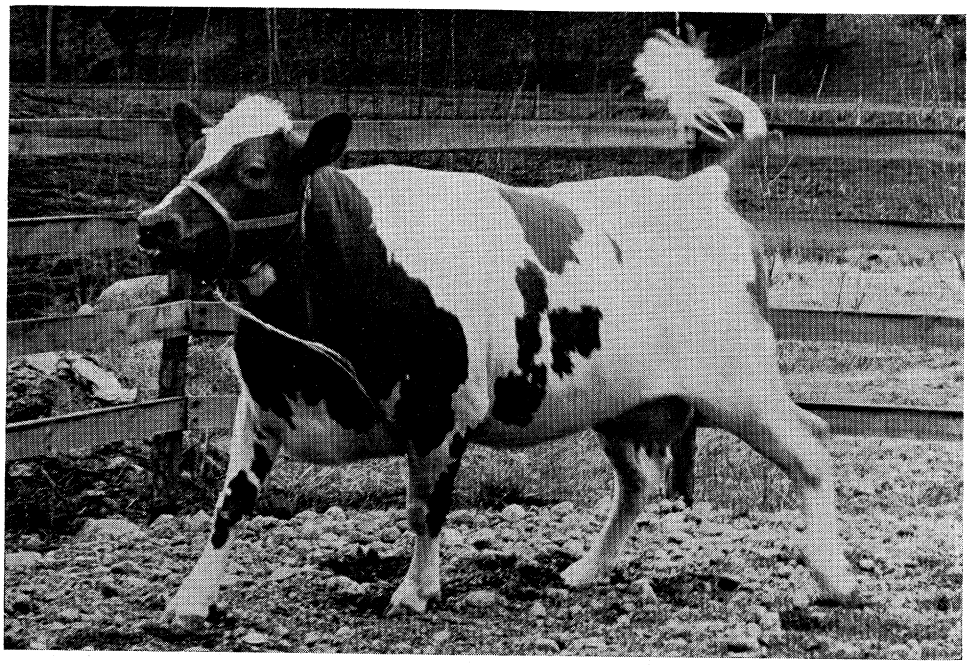


FIG. 15. Heifer-cow Lila after being on a feed poor in protein and rich in urea for 16 months.

feed rations of which have contained 45–49 % digestible true protein-N of the digestible total protein-N, and correspondingly less urea-N, have given lower milk yields due partly to a lower milk production capacity, partly owing to a less suitable ration composition (more grain, no sugar beet pulp). As the number of cows in our feeding experiments has been small, the real comparison between milk yields is of course questionable. As said before, groups of compounds other than true protein may influence the rise in milk production when nonpurified feed is used. We have found for instance in the blood serum of our test cows on purified, protein-free feed on the average only about 50 mg/100 ml cholesterol and 160 mg/100 ml total lipid, the corresponding values on normal feed and on low-protein experimental feed being three times higher. As far as I know such low cholesterol and lipid contents have been found with no other adult animal as with our test cows on protein-free feed. Photos of *cows 6* and *20* are presented in Figs. 14 and 15.

It is clear that experiments with a few cows only show the possibilities for dairy cows to utilize urea. Large-scale experiments for comparison of the effectiveness of protein and urea as nitrogen sources for dairy cows are needed using different carbohydrate sources. In any case, our experiments have shown that it is possible to reach a surprisingly high milk production using feed low in protein and rich in urea.

Large amounts of hemicellulose syrup prepared from wood have been used in our experiments of a practical nature, because we wanted to elucidate the usability of this quantitatively important component of wood in the feed of dairy cows. As the protein can be substituted by urea in such amounts that earlier seemed impossible, the central problem in the feeding of dairy cows is how to provide enough carbohydrates with high digestibility In tropical countries sugar cane molasses, sweet potatoes, and similar plants rich in starch give possibilities for considerable milk production.

After this paper was written I received a manuscript of H. R. Conrad, J. W. Hibbs and J. R. Staubus from Ohio Agricultural Research and Development Center, Department of Dairy Science, Wooster: *Guidelines for Increasing Urea Utilization in Rations for Dairy Cows*, in which successful experiments with dairy cows are described when large amounts of urea are used.

REFERENCES


1. HOLZSCHUH, W., AND H. WETTERAU. *Arch. Tierernaehr.* 15: 429, 1965.
2. LAND, H., AND A. I. VIRTANEN. *Acta Chem. Scand.* 13: 489, 1959.
3. LARSON, B. L. *J. Dairy Sci.* 48: 133, 1965.
4. LARSON, B. L., AND D. C. GILLESPIE. *J. Biol. Chem.* 227: 565, 1957.
5. MCNAUGHT, M. L., E. C. OWEN, K. M. HENRY AND S. K. KON. *Biochem. J.* 56: 151, 1954.
6. NEELIN, J. M. *J. Dairy Sci.* 47: 506, 1964.
7. NURMIKKO, V. *Ann. Acad. Sci. Fennicae, Ser. A II* 54: 1, 1954.
8. OLTJEN, R. R. *Proc. Cornell Nutr. Conf. Feed Manufacturers, 1967.*
9. SYVÄOJA, E.-L., AND A. I. VIRTANEN. *Enzymologia* 29: 205, 1965.
10. VÁRADY, J., K. BODA, I. HAVASSY, M. BAJO AND J. TOMÁS. *Physiologa Bohemoslov.* 16: 571, 1967.
11. VIRTANEN, A. I. *Biochem. Z.* 338: 443, 1963.
12. VIRTANEN, A. I. *Umschau* 63: 770, 1963.
13. VIRTANEN, A. I. *Nachr. Giessener Hochschulges.* 33: 73, 1964.
14. VIRTANEN, A. I. *Science* 153: 1603, 1966.
15. VIRTANEN, A. I. In: *Urea as a Protein Supplement*, edited by M. H. Briggs. Oxford: Pergamon, 1967, p. 185–212.
16. VIRTANEN, A. I. *Agrochimica* 11: 289, 1967.
17. VIRTANEN, A. I. *Neth. Milk Dairy J.* 21: 223, 1967.
18. VIRTANEN, A. I., AND M. LAMPILA. *Suomen Kemistilehti B* 35: 244, 1962.
19. VIRTANEN, A. I., AND H. LAND. *Acta Agral. Fennica* 94: 1, 1959.